Transmission risk predicts avoidance of infected conspecifics in Trinidadian guppies

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Summary

1. Associating with conspecifics afflicted with infectious diseases increases the risk of becoming infected, but engaging in avoidance behaviour incurs the cost of lost social benefits. Across systems, infected individuals vary in the transmission risk they pose, so natural selection should favour risk-sensitive avoidance behaviour that optimally balances the costs and benefits of sociality.
2. Here we use the guppy *Poecilia reticulata*-*Gyrodactylus turnbulli* host-parasite system to test the prediction that individuals avoid infected conspecifics in proportion to the transmission risk they pose.

3. In dichotomous choice tests, uninfected fish avoided both the chemical and visual cues, presented separately, of infected conspecifics only in the later stages of infection.
4. A transmission experiment indicated that this avoidance behaviour accurately tracked transmission risk (quantified as both the speed at which transmission occurs and the number of parasites transmitting) through the course of infection.
5. Together, these findings reveal that uninfected hosts can use redundant cues across sensory systems to inform dynamic risk-sensitive avoidance behaviour. This correlation between the transmission risk posed by infected individuals and the avoidance response they elicit has implications for the evolutionary ecology of infectious disease, and its explicit inclusion may improve the ability of epidemic models to predict disease spread.

Key-words effective contact rate (β); group-living; infectious disease avoidance behaviour; parasite transmission; redundant multimodal cues; risk-sensitive behaviour; social behaviour; social evolution.

Introduction

Social interactions between individuals influence infectious disease dynamics at the population level (Clay *et al.* 2009; Grear, Perkins & Hudson 2009; Aiello *et al.* 2016), so understanding factors affecting these interactions and how they change in the presence of disease will facilitate more accurate predictions of how diseases spread (Lloyd-Smith *et al.* 2005; Hawley *et al.* 2011; Paull *et al.* 2012; Aiello *et al.* 2016;

50 VanderWaal & Ezenwa 2016). Social animals associating with infected conspecifics
51 likely increase their risk of infection, particularly with directly transmitted disease-
52 causing organisms, and there is evidence from multiple taxa that they avoid doing so
53 (Goodall 1986; Kiesecker *et al.* 1999; Kavaliers *et al.* 2003; Behringer, Butler &
54 Shields 2006; Croft *et al.* 2011; Schaller 2011; Poirotte *et al.* 2017). For many
55 animals, such 'social barriers' to disease transmission may be as important as
56 immunological or physical ones (Loehle 1995; Schaller 2011; Zylberberg, Klasing &
57 Hahn 2013). However, engaging in avoidance behaviour incurs the cost of lost social
58 benefits (e.g. antipredator defence, foraging efficiency, mating opportunities: Seppälä,
59 Karvonen & Valtonen 2008; Croft *et al.* 2011; Schaller 2011).
60
61 The outcome of this trade-off may be determined by the probability contact with a
62 particular infected individual will result in transmission, or its 'infectiousness'.
63 Infectiousness is highly heterogeneous in natural populations: the vast majority of
64 transmission events involve a minority of infected individuals (Lloyd-Smith *et al.*
65 2005; Paull *et al.* 2012). How infectious an individual is depends on the
66 characteristics of its infection. For example, across a variety of systems the number of
67 parasites an individual is infected with, its 'infection load', is an important predictor
68 of the number of infectious particles it releases, and hence the transmission risk it
69 poses to uninfected conspecifics (e.g. Matthews *et al.* 2006; Aiello *et al.* 2016;
70 Stephenson *et al.* 2017). As well as variation between individuals, a single
71 individual's infection load and hence infectiousness is, for many disease systems,
72 likely to change through the course of infection (Poulin 2007; Schmid-Hempel 2011).
73 Infection duration also encompasses variation in the strength of the host's immune
74 response, symptoms and behaviour, as well as the demography of the infecting

parasites and their ability to transmit and establish infections on new hosts (Scott & Anderson 1984; Schmid-Hempel *et al.* 1999; Bakke, Cable & Harris 2007; Chase-Topping *et al.* 2008; Charleston *et al.* 2011; Therese & Bashey 2012; Fraser *et al.* 2014; Aiello *et al.* 2016). Given this heterogeneity, natural selection should favour the evolution of mechanisms that maximize the cost-benefit balance of association and avoidance, such as avoidance behaviour that is sensitive to the transmission risk posed by individual conspecifics.

The prediction that uninfected individuals mitigate the risk posed by infectious individuals by modulating their own avoidance behaviour can be formalized using an epidemiological modelling framework. In such models, the effective contact rate, β, is the product of the contact rate between infected and uninfected individuals (behavioural component of β, β_c) and the transmission rate per contact, which is often driven by the infected hosts' response to the parasites, mediated by infection load (physiological component of β, β_p; Anderson & May 1991; Lloyd-Smith *et al.* 2005; Hawley *et al.* 2011; VanderWaal & Ezenwa 2016). Historically, models have assumed homogeneous population mixing and transmission risk, i.e. mean field estimates of β_c and β_p, but this typically leads to overestimated transmission rates (Keeling & Grenfell 2000). More recent work has demonstrated that incorporating empirical estimates of heterogeneity in both β_c and β_p improves model fit to natural disease dynamics (see Aiello *et al.* 2016 and references therein), but that β_c and β_p may themselves co-vary has been largely ignored. However, this co-variation has potentially powerful implications for disease dynamics. For example, using a simple modelling framework, Hawley *et al.* (2011) showed that behaviourally-mediated co-variation in β_c and β_p, such as risk-sensitive avoidance of infectious conspecifics, can

mean the difference between a parasite invading a host population or fading out. Despite this, empirical tests of how β_c and β_p co-vary in natural systems are still lacking (Hawley *et al.* 2011; VanderWaal & Ezenwa 2016).

We used the guppy *Poecilia reticulata-Gyrodactylus turnbulli* host-parasite system to experimentally test for risk-sensitive avoidance of infectious conspecifics. *G. turnbulli* is an ectoparasitic monogenean that reproduces on the host's skin with a generation time of 24 hrs and is transmitted directly through close contact between socially interacting hosts (Stephenson *et al.* 2015a). *Gyrodactylus* spp. parasites are the most prevalent multicellular parasites in wild guppy populations (Stephenson *et al.* 2015a), and are associated with reduced guppy body condition (Stephenson, van Oosterhout & Cable 2015b), attractiveness (Kennedy *et al.* 1987), and survival (van Oosterhout *et al.* 2007; Stephenson *et al.* 2016). The ability to recognize and avoid infected individuals is therefore likely to be under strong selection and there is some evidence that it occurs; the presence of infected conspecifics reduces shoal cohesion in semi-natural conditions (Croft *et al.* 2011). However, the loss of shoal cohesion as a result of this infection avoidance behaviour carries a cost: less cohesive fish shoals are more vulnerable to predation (Seppälä, Karvonen & Valtonen 2008). If guppies balance this trade-off by employing risk-sensitive avoidance of infected conspecifics, avoidance should be positively correlated with infection duration: infection load initially increases over the course of infection, and is an important predictor of transmission risk (Stephenson *et al.* 2017).

Beyond favouring the evolution of risk-sensitive behaviour, natural selection should favour the use of cues appropriate to the sensory environment. For example, in static

125 water bodies, chemical cues may provide reliable information, but turbidity may limit
126 the usefulness of visual cues; correspondingly, tadpoles use chemical but not visual
127 cues to avoid infected conspecifics (Kiesecker *et al.* 1999). By contrast, in habitats
128 characterized by dynamic sensory environments selection should favour the use of
129 multiple sensory modalities to detect and respond to redundant cues (i.e. those that
130 elicit the same response in receivers when presented in isolation; Partan & Marler
131 2005). Such cue redundancy is most likely to evolve in habitats in which no single
132 sense is continuously informative. Rivers, such as those inhabited by guppies,
133 experience turbulent flow and turbidity; as a result, visual and chemical cues elicit
134 redundant risk-sensitive antipredator behaviour in several riverine fishes (e.g. the
135 naked characin, *Gymnocharacinus bergi*; see Cordi, Ortubay & Lozada 2005).
136 Guppies may use similarly redundant visual and chemical cues in risk-sensitive
137 infection avoidance behaviour. Previous work has shown that they are able to use
138 chemical cues to monitor temporally variable physiological characteristics in
139 conspecifics (reproductive status: Brask *et al.* 2012; disease: Stephenson & Reynolds
140 2016), and have excellent vision (Anstis, Hutahajan & Cavanagh 1998). However,
141 visual cues of infection may provide a general 'sickness' cue and include behaviour,
142 which host animals are able to modify in the short term to conceal their disease (e.g.
143 Lopes *et al.* 2012). Chemical cues potentially provide more honest, less easily
144 manipulable information about health, which may also be specific to the disease-
145 causing agent: guppies may therefore respond differently to cues across these sensory
146 modalities.
147
148 We here test the prediction that social hosts display risk-sensitive avoidance of
149 infected conspecifics that pose the highest risk of transmission. We presented

uninfected 'test' guppies with a dichotomous choice between the cues (visual or chemical, presented separately) of *G. turnbulli*-infected and uninfected conspecific 'stimulus' fish. Uninfected guppies avoided both chemical and visual cues of infected conspecifics only in the later stages of infection. Models developed from a transmission experiment using this system (Stephenson *et al.* 2017) predicted that both transmission speed and the number of parasites transmitting increase through the course of the infection on the stimulus fish. Indeed, days on which the predicted risk was highest were those on which avoidance was strongest. These results comprise the first demonstration that infection avoidance behaviour is sensitive to present infection risk (β_c and β_p are negatively correlated), and therefore highlight a potentially important and under-studied source of variation in infectious disease transmission.

Materials and methods

Host and parasite origin and maintenance

We used wild caught guppies and their laboratory-bred descendants from the Caura River, Trinidad, and a single strain of the parasite *Gyrodactylus turnbulli* (*Gt3*). Guppies were housed at low densities in 70 L aquaria at 24±1°C, on a 12 h light: 12 h dark lighting schedule (overhead fluorescent lighting), and fed daily on Aquarian® flakes, supplemented with *Artemia* and bloodworm. *Gt3* was originally isolated from an ornamental guppy and has been maintained on inbred ornamental stocks ('culture fish') in the laboratory since 1997.

Chemical and visual cue production

We used F1 laboratory-bred virgin females to produce the chemical and visual cues of infection. These 'stimulus pairs' (uninfected *vs.* infected, $n = 28$ pairs) were size-

matched ±1 mm. Recently killed infected *Gt3* culture fish were placed in close proximity to the anesthetized (0.02% tricaine methanesulfonate; MS222; PHARMAQ Ltd., Fordingbridge, UK) stimulus fish until two parasites had transferred, as observed under a dissecting microscope and fibre optic illumination. The stimulus fish were revived and housed individually in 1 L tanks, and the number of parasites infecting each was counted under anaesthetic every other day. As a handling control, uninfected stimulus fish were also anesthetized and held individually in 1 L tanks. All tanks were maintained under standard conditions and received 100% water exchanges every other day. We exclusively used female guppies as stimulus fish because male guppies typically have complex and highly polymorphic colour patterns that affect how both male and female conspecifics respond to them (reviewed in e.g. Houde 1997). By only using females, therefore, we avoided the substantial challenge of standardising male colour patterns among and between pairs.

The pairs of infected and uninfected fish were used to produce chemical stimuli for the behavioural trials. Due to a change in experimental design, chemical cues were produced either in batches or pairs. During the production of each batch, five fish were held individually, each in 500 ml of dechlorinated water in food grade plastic containers for 24 h. Fish were not fed during this isolation. These 500 ml fish conditioned water samples were then mixed and frozen in 150 ml aliquots at -20°C. During the production of paired chemical cues the same protocol was followed except that the samples from each stimulus fish were kept separate (see Appendix S1: Table S1 for more details).

Avoidance behaviour experiment

We exposed uninfected guppies ('test fish') to the chemical (n = 87) and visual (n = 83) cues of the stimulus pairs. All test and stimulus fish were unfamiliar to one another, i.e. they had never been in the same or adjacent stock tanks. We manipulated the length of time the infected stimulus fish had been infected, and measured the avoidance behaviour elicited in the test fish. We used a 30 × 60 cm tank, filled to 5 cm water depth (Appendix S1: Fig. S1). At one end of the tank we placed two glass cylinders with adjacent Nalgene® tubing, separated by an opaque barrier. At the other end was a settling compartment (10 × 30 cm), separated from the test arena by a removable opaque barrier. For the chemical cue trials, cues were introduced via the Nalgene® tubing at 10 ml/min, maintained by flow meters (MMA-35, Dwyer Instruments, High Wycombe, UK). Test fish of both sexes were taken from the wild-caught parental and F2 generations (see Appendix S1: Table S1) and tested individually. Fish acclimatized in the settling compartment for 10 min. For the visual cue trials, stimulus pairs were placed in the glass cylinders, one fish per cylinder, before this acclimatization period. The glass cylinders were entirely watertight and washed inside and out between trials with 70% ethanol and clean water: no chemical cues of the stimulus pair could have been detected by the test fish during the visual cue trials. In chemical trials, the flow of chemical cues (infected *vs.* uninfected) was started two min before the end of acclimatization. The barrier was lifted remotely *via* a pulley system at the end of the acclimatization period, and a 10 min test period began when the fish crossed into the test arena. After each trial the tank and components were rinsed with 70% ethanol and clean water. The sex of the test fish and the side of the tank that received the cue of infected conspecific were changed between trials according to a Latin square design. All behavioural trials were video recorded for later analysis using JWatcher™ 1.0 (www.jwatcher.ucla.edu).

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226 We used different measures of association for the two senses to accommodate
227 inherent differences between them: chemical cues could be detected across the whole
228 side of the tank, while visually mediated preference is typically measured in time
229 spent in proximity to the stimulus fish (Houde 1997). For chemical cue trials,
230 therefore, we used the proportion of the 10 min test period that test fish spent on the
231 side of the tank that received the cue of the uninfected fish. For visual cue trials we
232 used the proportion of time test fish spent on the side of the 'end zone' next to the
233 uninfected fish out of the total time (out of the 10 min test period) that test fish spent
234 in the end zone (Appendix 1: Fig. S1).

235

236 *Predicting transmission risk*

237 To predict the transmission risk posed by the infected stimulus fish on each day of
238 infection on which they were used as stimuli, we used models built on data from a
239 transmission experiment using this system (for detailed methods and results see
240 Stephenson *et al.* 2017). In brief, we experimentally infected parasite-naïve
241 laboratory-bred females descended from guppies caught in the lower Aripo river,
242 Trinidad ('donors', $n = 60$), using the methods and *Gt3* parasite strain described
243 above. We exclusively used female fish in this experiment to minimise variation in
244 transmission attributable to the differences in behaviour between male and female
245 guppies. We housed the donors individually in 1 L tanks and allowed them to develop
246 natural variation in infection loads. On days 5 and 12 of infection, parasite-naïve
247 female 'recipients' were size-matched to the donors±2 mm and added to the tanks.
248 The number of *G. turnbulli* parasites on both donor and recipient was recorded daily.
249 Once transmission had occurred, the recipient was removed from the tank. We thus

observed 105 transmission events, and used the data to construct Generalized Linear Mixed Models (GLMMs) explaining variation in how quickly transmission occurred ('transmission speed') and how many parasites transmitted ('transmission load'). The best-supported model for transmission speed included only the donor's infection load at the time of transmission, and that for transmission load included donor infection load, donor infection integral (i.e. the area under the curve of its infection load over time), and the day of infection of the donor (Stephenson *et al.* 2017). Using these models and the infection load, infection integral and day of infection on which they were used, we calculated the model predictions of the transmission speed and load of the stimulus fish in the behavioural experiment.

Data analysis

We analysed the data using R 3.3.1 (R Core Team 2016), and provide the data, script and output in Appendix S1. We used the proportion of time the test fish spent associated with the uninfected stimulus fish cue (i.e. avoiding the infected stimulus fish cue) as the response variable in a GLMM (beta error distribution with logit link function in the glmmADMB package; Fournier *et al.* 2012). As fixed effects, we included the day of infection and infection integral (i.e. the area under the curve of its infection load over time) of the stimulus fish; test fish sex and standard length; the cue type used (chemical or visual) and the side of the tank in which the cue of infected conspecific was placed (to test for any side bias). We also included the year in which the tests were conducted, which encompassed changes in test fish generation (wild-caught parental *vs.* laboratory-bred F2) and in stimulus production method (batch *vs.* pair; see Appendix S1: Table S1 for more details). We included the two-way interactions between test fish sex, cue type (visual or chemical), day of infection and

infection integral about which we had a priori hypotheses. The identity of the stimulus pair used in a trial was included as a random term as each was used on multiple days. The full output of this model is presented in Appendix S1.

We used two GLMMs to test whether the predicted transmission speed and transmission load of the stimulus fish increased through time (both Gamma error family, log link function in lme4; Bates *et al.* 2015). We included day of infection as a fixed effect, and the stimulus pair identity as a random effect to control for the fact that each was used on multiple days. These data are values predicted from a statistical model and therefore have error associated with them. In order to investigate whether this error affected the conclusions we are able to draw from this analysis, we reran the GLMMs using both high and low estimates of the predicted values (value±1 standard error).

Results

The full output and model fits for all models are given in Appendix S1. The length of time the stimulus fish had been infected (day of infection) was the only variable that explained variation in the proportion of time test fish spent avoiding the infected stimulus fish, with test fish only avoiding stimulus fish in the later stages of infection ($\chi^2 = 9.84$, $P = 0.0017$; Fig. 1). There was no significant effect of cue type, or its interaction with day of infection, indicating redundancy between the visual and chemical cues. The predicted transmission speed (predicted values: $t_{92} = -2.15$, $P = 0.032$; low estimate: $t_{92} = -2.61$, $P = 0.009$; high estimate: $t_{92} = -1.68$, $P = 0.093$) and transmission load (predicted values: $t_{92} = 6.59$, $P < 0.0001$; low estimate: $t_{92} = 4.23$, P

299 <0.0001; high estimate: $t_{92} = 4.81$, $P < 0.0001$) of the stimulus fish increased through
300 the course of their infection (Fig. 2).
301
302 In post-hoc tests investigating the apparent threshold at day 15 of infection we found
303 no difference between test fish response to chemical and visual cues (main effect) or
304 how visually and chemically mediated behaviour changed depending on the duration
305 of the infection of the stimulus fish (pre vs post day 15 interaction with cue type),
306 again indicating redundancy between these multimodal cues. Guppies marginally but
307 significantly preferred (i.e. spent more than 50% of the time associating with)
308 conspecifics infected for fewer than 15 days over uninfected counterparts (mean±SE =
309 0.55±0.02, $t_{122} = 2.56$, $P = 0.012$), but strongly avoided those infected for longer than
310 15 days (i.e. spent less than 50% of the time with; mean±SE = 0.40±0.03, $t_{46} = -3.16$,
311 $P = 0.0027$). Pre- and post-15 day stimulus fish elicited significantly different
312 responses in test fish ($\chi^2 = 15.15$, $P < 0.0001$). Moreover, post-day 15 infection
313 stimulus fish had significantly higher predicted transmission loads (predicted values:
314 $t_{92} = 3.23$, $P = 0.0012$; low estimate: $t_{92} = 165.6$, $P < 0.0001$; high estimate: $t_{92} =$
315 205.6, $P < 0.0001$), but not speeds (all $P > 0.05$), than pre-day 15 stimulus fish.
316

317 ## Discussion

318 We tested whether natural selection has driven the evolution of infection avoidance
319 behaviour that could potentially optimally balance the costs and benefits of sociality.
320 In a dichotomous choice test, uninfected guppies avoided both the visual and chemical
321 cues, presented separately, of *Gyrodactylus turnbulli*-infected conspecifics only in the
322 later stages of infection (Fig. 1). Predictions of the transmission risk posed by these
323 infected conspecifics from models built on data from a transmission experiment using

this system (Stephenson *et al.* 2017) illustrated that this avoidance behaviour tracked transmission risk through time, such that those that posed the highest predicted risk were most strongly avoided (Fig. 2). Our data represent unique empirical evidence that the two components of the effective contact rate β (contact rate, β_c, and infectiousness, β_p) co-vary quantitatively, rather than as a binary comparison of infected and uninfected individuals.

Both chemical and visual cues for avoidance behaviour may be primarily derived from the host and its response to the parasite, rather than from the parasite itself. This suggestion is based on two observations. First, stimulus fish infection duration, rather than infection load, was the most important predictor of avoidance behaviour in this study. Second, guppies that have imprinted on the chemical cues of conspecifics experiencing *G. turnbulli*-induced disease, but that have been parasite-free for over a month, preferentially associate with the chemical cues of conspecifics in the late stages of *G. turnbulli* infection (Stephenson & Reynolds 2016). There thus appears to be a host-derived chemical cue of *G. turnbulli*-induced disease that elicits behavioural responses in conspecifics. Parasite-derived cues may not elicit a response because directly transmitted parasites are under strong selection to conceal their presence on the host, thereby increasing their chances of transmitting to new hosts (Poulin 2007). Indeed, malaria parasites strategically control the emission of chemical cues to maximize their fitness, attracting vectors particularly strongly when they are ready to transmit (Cornet *et al.* 2013; De Moraes *et al.* 2014).

Infectious hosts should also be under strong selection to disguise their infection in order to continue benefitting from group living, and to increase their relative fitness

by transmitting parasites to unrelated group mates. In other systems hosts conceal pathology and sickness behaviour (Lopes *et al.* 2012), and early in infection the guppies in our experiment also appear to do so successfully, and are even marginally more attractive than their uninfected counterparts. This counterintuitive observation may be due to the infected stimulus fish interacting more with the test fish, or having a generally higher activity level than the uninfected fish; infected fish tend to initiate more social interactions in semi-natural conditions (Croft *et al.* 2011).

The many potential cues of infection likely become increasingly difficult to suppress through the course of infection: in our data, a critical threshold in cue composition or concentration appears to be reached after 15 days of infection. One component may be alarm cue, a chemical released from fish skin damaged during predation events and infection (Poulin, Marcogliese & McLaughlin 1999), which elicits avoidance behaviour in guppies and many other species (Brown *et al.* 2009 and references therein). Other chemical cues may be related to epithelial cell composition or mucous chemistry, both of which change during the course of gyrodactylid infection (Buchmann & Lindenstrøm 2002; Gheorghiu, Marcogliese & Scott 2012). The parasite itself may use chemical cues from the host, or conspecifics, to determine when the benefits of transmission outweigh the risks (Stephenson 2012; Stephenson *et al.* 2017): such cues may therefore accurately reflect the real-time probability of parasite transmission. The visual cues of infection also become more obvious as the infection progresses. For example, guppies may display clamped fins, paleness, and difficulty swimming (Kennedy *et al.* 1987). Additionally, during later stages of infection gyrodactylid-infected guppies attempt to ‘rub up’ against shoal-mates (Croft *et al.* 2011). This abnormal behaviour itself, and the opportunity it provides shoal-

mates to sample the host's chemical and visual cues at close range, potentially explains their observed avoidance by conspecifics in semi-natural conditions (Croft *et al.* 2011). Indeed, it is likely to be the abnormality of these cues, rather than what they signify, that guppies avoid (Stephenson & Reynolds 2016).

If the cues of infection are indeed host-derived and independent of infection load, as our data suggest, the infection avoidance behaviour they mediate could be widespread in natural populations despite the relatively low infection loads observed in field surveys (Stephenson *et al.* 2015a). Further, while the cues in our experiment were presented separately, in natural settings guppies are likely often in receipt of both. Together, they could have an effect equal to that of either cue alone or the response could be greater (Partan & Marler 2005); guppies are more attentive to visual cues when in receipt of chemical cues (Stephenson 2016). In avoiding infected individuals, guppies in natural populations also benefit from avoiding predators that might use the same cues to find relatively easy prey (Stephenson *et al.* 2016). Indeed, ostracizing infected individuals, thereby facilitating their capture by predators, may have the added benefit of reducing population level parasite prevalence and intensity (Packer *et al.* 2003), and thus the per capita infection risk. In a further contrast with the natural setting we constrained the stimulus fish in this experiment, but previous work on this and other systems suggests that infection may increase or decrease their attempts to interact (Croft *et al.* 2011; Lopes, Block & König 2016). Future work should elucidate how the behaviour of infected and uninfected hosts interacts with the infectiousness of infected hosts in driving disease transmission.

Our results highlight the importance of accounting for the feedback between host and parasite during the infection process in modelling the spread of infectious diseases (Ezenwa *et al.* 2016): a particular pitfall if basing such inference on empirically derived static social networks of uninfected animals (e.g. references in Rushmore, Bisanzio & Gillespie 2017). Modelling approaches provide one solution to this issue by incorporating the uncertainty associated with the co-dynamics of network structure and infection into static models, offering insight where the interplay is an empirical unknown (Silk *et al.* 2017). However, we have shown that disease can have a quantitative, non-linear effect on the contact behaviour of social animals, indicating that using dynamic models explicitly incorporating this feedback between infection and behaviour will likely improve predictions (Farine 2017). The relationship between β_c and β_p may also drive evolutionary change in both host and parasite. For example, heritable variation between uninfected hosts in their ability to avoid infected conspecifics (Zylberberg, Klasing & Hahn 2013), and between infected hosts in their ability to transmit the parasite (Boots *et al.* 2012), can shape the evolution of host defence mechanisms. Additionally, disease transmission and the interactions between infected and susceptible hosts drive the evolution of parasite virulence (e.g. Lion & Boots 2010). In light of its potentially profound importance for the evolutionary ecology of disease, further empirical and theoretical consideration of the relationship between β_c and β_p and the factors affecting it are sorely needed.

Data Accessibility

Data supporting the results will be archived in the Dryad repository and the data DOI will be included at the end of the article.

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Authors' contributions

J. F. S. conceived the study, designed and conducted the behavioural experiment, analysed all data, interpreted the results, wrote and, with S. E. P., revised the paper. S. E. P. and J. C. designed and conducted the transmission experiment. All authors gave final approval for publication, and agree to be accountable for the accuracy and integrity of their work.

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Supporting Information

The following supporting information is available for this article online:

642 Appendix S1. This file contains supplementary methodological details, as referred to
643 in the methods (Figure S1 and Table S1). It also provides the code and full output of
644 all analyses described in the main text.
645

646 # Figure legends

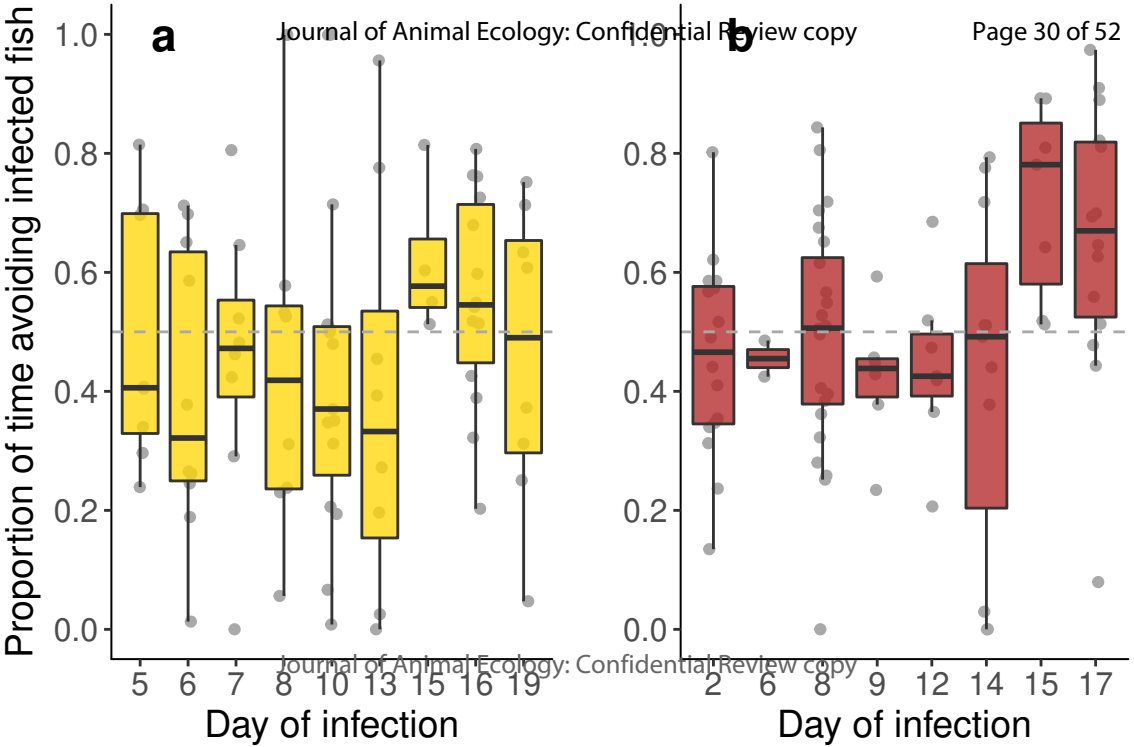
647

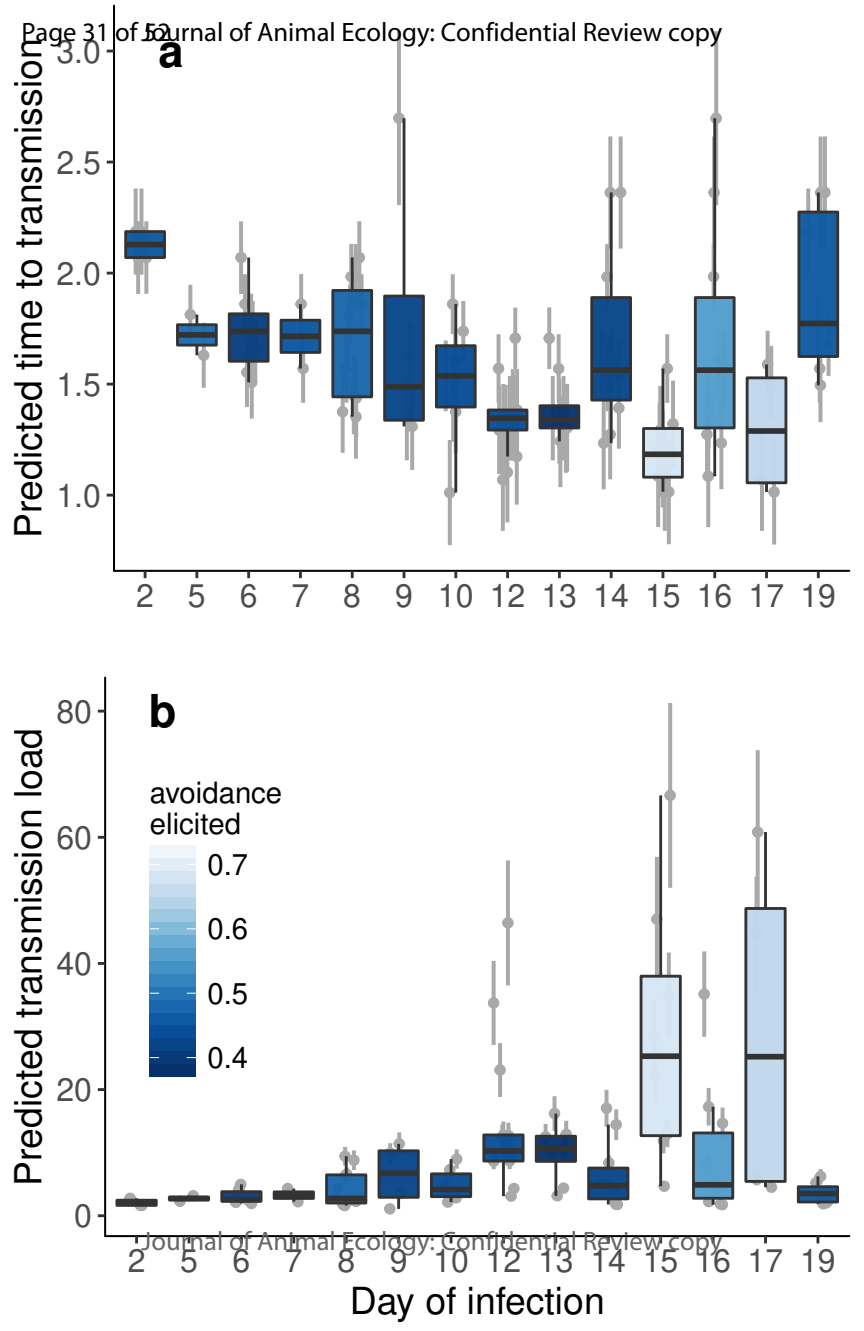
648 **Fig. 1.** Uninfected guppies avoided *Gyrodactylus turnbulli*-infected conspecifics only
649 when these were in the later stages of infection, based on both visual (a) and chemical
650 (b) cues. The points give the raw data, thick lines the median, boxes the first and third
651 quartiles, and whiskers extend to the largest and smallest value within 1.5 × the
652 interquartile range.

653

654 **Fig. 2.** The predicted speed (in days) at which transmission would occur (a), and the
655 number of parasites transmitting (b) from the stimulus fish increased through the
656 course of infection, and covaried with the avoidance behaviour they elicited. The
657 points give the values (± 1 standard error) predicted by models built on data from 105
658 transmission events (from the experiment presented in Stephenson *et al.* 2017), and
659 using the infection load, infection integral (i.e. the area under the curve of its infection
660 load over time) and day of infection of the stimulus fish in the present experiment.
661 Thick lines denote the median values, boxes the first and third quartiles, and whiskers
662 extend to the largest and smallest value within 1.5 × the interquartile range. The
663 shading of the boxes denotes the mean behavioural avoidance elicited by the stimulus
664 fish on each day of infection, as given by the scale bar (raw data in Fig. 1). One
665 outlying data point (with a predicted transmission load of 90) has been omitted from
666 (b) for clarity and the analysis to facilitate model convergence.

667





Appendix S1: Transmission risk predicts avoidance of infected conspecifics

J. F. Stephenson, S. E. Perkins, J. Cable

In this paper we explore how an individual's avoidance behaviour is determined by the transmission risk posed by infected conspecifics, and how visual and chemical cues may be used to detect changes in transmission risk. This document is composed of two main sections. In the first, we present Fig. S1 and Table S1, which provide more details on the methods we employed. In the second, we present further details of the three steps involved in the data analyses. First, analyses of behavioural data show that uninfected guppies *Poecilia reticulata* spend less time with conspecifics infected with a directly transmitted monogenean *Gyrodactylus turnbulli*, but only during the later stages of infection. In the second, we use models explaining variation in the speed at which transmission occurs, and the number of parasites transmitting (constructed using data from this system, published in Stephenson et al 2017, Phil. Trans. Roy. Soc. B.), to predict the transmission risk, both in terms of speed and load, posed by the stimulus fish used in the behavioural experiment. We use these predicted values to explore whether variation in transmission risk might explain the pattern observed in the behavioural data. Finally, we present post-hoc tests investigating an apparent threshold at day 15 of infection on the stimulus fish. Further details on the methods of both experiments and our interpretation of the results can be found in the main text.

Supplementary methods: Figure S1 and Table S1

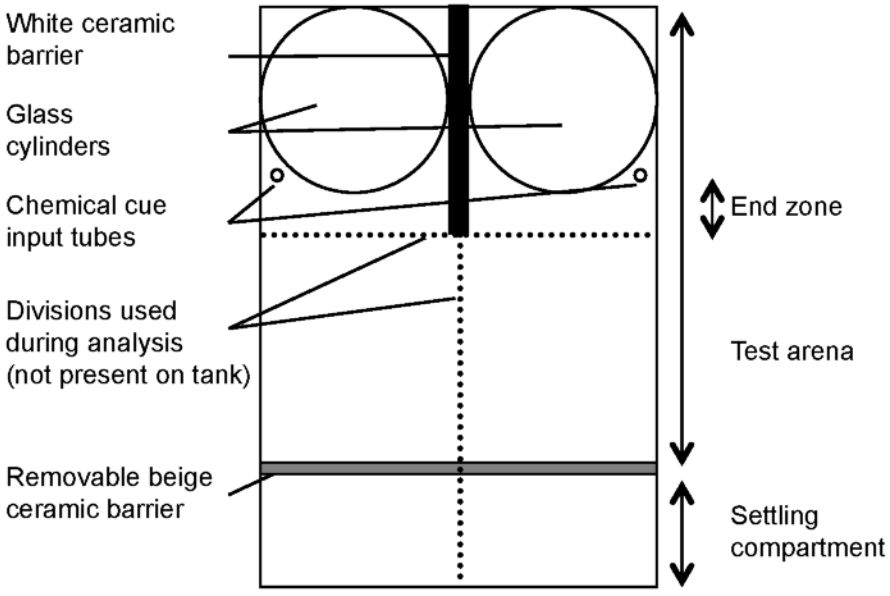


Fig. S1 The choice chamber used to test for behavioural responses of guppies to chemical and visual cues of infection in conspecifics. The dotted lines were not present on the tank, but delineate the zones and sides of the tank used during video analysis.

Table S1. Visual and chemical cue production and use during behavioural trials to test for responses of guppies to *Gyrodactylus turnbulli* infection in conspecifics. Stimulus fish were first generation laboratory-bred female offspring of wild caught guppies from Trinidad and were sexually mature virgins. F2 test fish were second-generation laboratory-bred sexually mature virgins of both sexes. Data are presented for the stage of infection rather than for each day for brevity (the 'early' stage of infection was up to Day 11).

Year	Cue type	Stage of infection	Cue production method	No. of stimulus pairs or batches	Days of infection on which the stimulus was used	Stimulus fish (females only)	Mean no. of parasites on the infected stimulus fish	Test fish (both sexes)	Mean no. of trials conducted with each pair or batch	Total no. of trials
2013	Visual	Early	Pairs	7	5, 6, 7, 8, 10	F1	12.5	Wild caught	5.1	36
		Late		7	15, 16, 20		63.5		4.3	30
	Chemical	Early	Batches	3	2, 8		9.4		13.3	40
		Late		1	17		83		14	14
2014	Visual	Early	Pairs	11	6, 8, 10		32.4	F2	1.2	13
		Late		23	13, 16, 19		23.3		1	24
	Chemical	Early		5	6, 9		16.5		1.6	8
		Late		15	12, 14, 15, 17		57.7		1.3	20

Data analyses

```
df1<-read.csv('DatasetS2.csv')
df2<-read.csv('DatasetS3.csv')

df1$iL<-as.numeric(as.character(df1$iL))
df1$uL<-as.numeric(as.character(df1$uL))
df1$AUC<-as.numeric(as.character(df1$AUC))
df1$year<-as.factor(df1$year)
df1$speedmax<-as.numeric(as.character(df1$speedmax))
df1$speed<-as.numeric(as.character(df1$speed))

require('lme4')
require('car')
require('MuMIn')
require('itsadug')
require('ggplot2')
require('gridExtra')
require('arm')
require('glmmADMB')
require('visreg')
require('MASS')
require('lsmeans')
require('ResourceSelection')
```

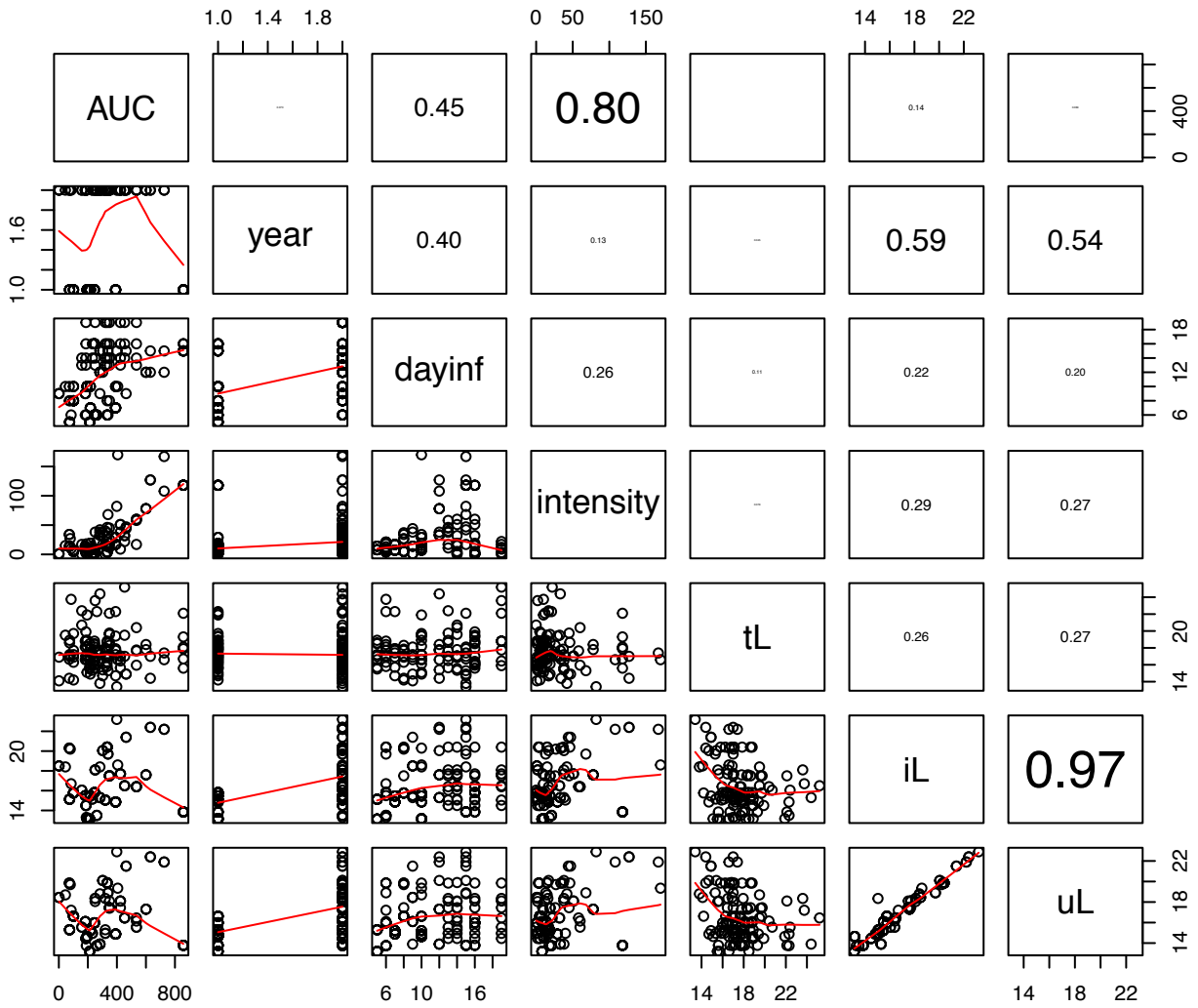
Avoidance behaviour changes through time, and is based on redundant visual and chemical cues

For this analysis we used the data in the archived file 'DatasetS2.csv', which includes the following variables:

- **resp:** The proportion of time test fish spent associated with the cue of infected conspecific - our response variable.
- **pair:** The identity of the pair of stimulus fish used in a trial - a random effect controlling for repeated measures. Those labelled with a letter were batch-produced cues.
- **dayinf:** The day of infection on which cues from the stimulus pair were created (chemical) or used (visual).
- **intensitymax:** The number of parasites on the infected stimulus fish on the day on which the stimulus was created (chemical) or used (visual). For trials in which a batch-produced chemical cue was used, we took the maximum individual intensity within that batch.
- **AUC:** The area under the curve of the stimulus fish's infection load over the course of its infection up to day 18 - a measure of its resistance, or ability to limit parasite growth. We have previously shown that transmission is affected by the resistance of the donor (details in the main text), and therefore tested if resistance of an infected conspecific affected how uninfected conspecifics responded to it.
- **sex:** The sex of the test fish (N.B. all stimulus fish were female).
- **tL, iL, uL:** Standard length (mm) of the test fish, infected stimulus fish and uninfected stimulus fish, respectively.
- **sense:** The sensory modality of the cue - c for chemical, v for visual.
- **infecinput:** The side of the test tank on which the cue of infection was placed, to test for side bias.

- **year:** The year of the experiment in which the trial was conducted. This factor encompasses changes in the generation of fish used, and the method of chemical cue production (batch vs paired).

```
# This function is from 'Mixed effects models and extensions
# in ecology with R'. (2009).Zuur, AF et al. Springer.
panel.cor <- function(x, y, digits = 2, prefix = "", cex.cor,
    ...) {
    usr <- par("usr")
    on.exit(par(usr))
    par(usr = c(0, 1, 0, 1))
    r <- abs(cor(x, y))
    txt <- format(c(r, 0.123456789), digits = digits)[1]
    txt <- paste(prefix, txt, sep = "")
    if (missing(cex.cor))
        cex.cor <- 0.8/strwidth(txt)
    text(0.5, 0.5, txt, cex = cex.cor * r)
}

pairs(~AUC + year + dayinf + intensity + tL + iL + uL, data = df1,
    lower.panel = panel.smooth, upper.panel = panel.cor, na.action = na.omit)
```



This plot shows that AUC and intensity were highly correlated. We decided to include AUC in our analyses,

and remove intensity. Apart from this, no pairs of the the continuous variables we were interested in showed a correlation of over 0.6, except for iL and uL (which is unsurprising given the infected and uninfected stimulus fish were size-matched). We therefore proceeded with the generalised linear mixed model including these factors, as below.

```
modb <- glmmadmb(resp ~ dayinf + sense + sex + tL + year + infecinput +
    sense:dayinf + sex:dayinf + sex:sense + AUC + AUC:sense +
    AUC:sex + (1 | pair), data = df1, family = "beta")

summary(modb)
```

```
##
## Call:
## glmmadmb(formula = resp ~ dayinf + sense + sex + tL + year +
##     infecinput + sense:dayinf + sex:dayinf + sex:sense + AUC +
##     AUC:sense + AUC:sex + (1 | pair), data = df1, family = "beta")
##
## AIC: -14.5
##
## Coefficients:
##                Estimate Std. Error z value Pr(>|z|)
## (Intercept)   -0.661265   1.135500   -0.58    0.560
## dayinf         0.042632   0.026944    1.58    0.114
## sensev         0.257380   0.792860    0.32    0.745
## sexm          -0.227205   0.490650   -0.46    0.643
## tL            -0.014686   0.049238   -0.30    0.766
## year2014      -0.252935   0.368760   -0.69    0.493
## infecinputr    0.179579   0.158260    1.13    0.256
## AUC            0.000987   0.001287    0.77    0.443
## dayinf:sensev  0.041577   0.042277    0.98    0.325
## dayinf:sexm    0.012974   0.032562    0.40    0.690
## sensev:sexm    0.273594   0.360600    0.76    0.448
## sensev:AUC    -0.002756   0.001640   -1.68    0.093 .
## sexm:AUC      -0.000176   0.000659   -0.27    0.790
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Number of observations: total=170, pair=48
## Random effect variance(s):
## Group=pair
##             Variance StdDev
## (Intercept)   0.8958 0.9464
##
## Beta dispersion parameter: 3.8191 (std. err.: 0.46928)
##
## Log-likelihood: 22.2491
```

```
Anova(modb)
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: resp
##              Df  Chisq Pr(>Chisq)
## dayinf        1 9.8395   0.001708 **
## sense         1 0.1660   0.683732
## sex           1 0.0628   0.802113
```

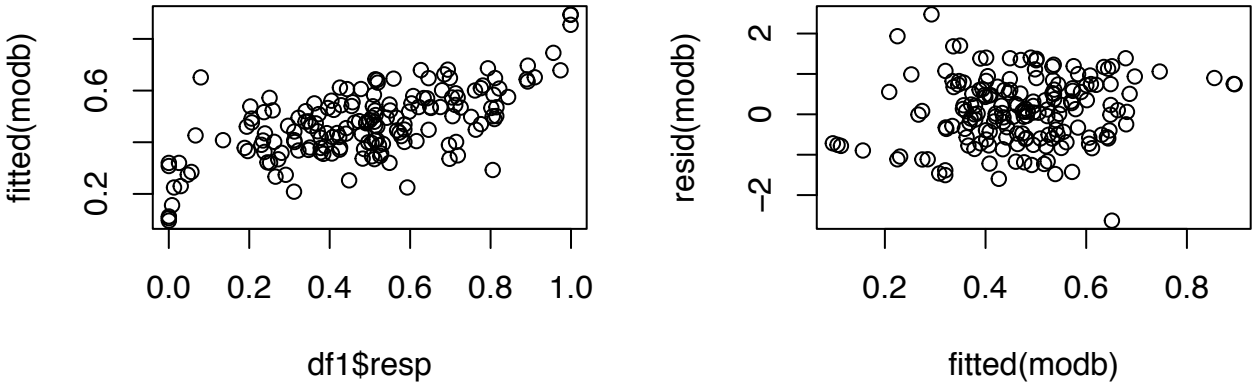
```
## tL            1 0.0890   0.765507
## year          1 0.4705   0.492773
## infecinput    1 1.2876   0.256498
## AUC           1 0.7917   0.373572
## dayinf:sense  1 0.9672   0.325388
## dayinf:sex    1 0.1588   0.690305
## sense:sex     1 0.5757   0.448021
## sense:AUC     1 2.8251   0.092801 .
## sex:AUC       1 0.0709   0.789972
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

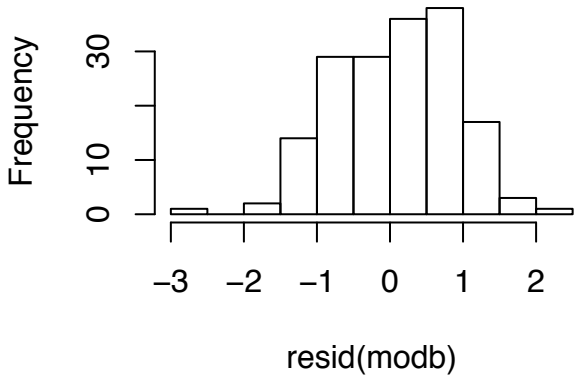
```
# this function tests for overdispersion.  It's from
# http://glmm.wikidot.com/faq
overdisp_fun <- function(model) {
    ## number of variance parameters in an n-by-n
    ## variance-covariance matrix
    vpars <- function(m) {
        nrow(m) * (nrow(m) + 1)/2
    }
    model.df <- sum(sapply(VarCorr(model), vpars)) + length(fixef(model))
    rdf <- nrow(model.frame(model)) - model.df
    rp <- residuals(model, type = "pearson")
    Pearson.chisq <- sum(rp^2)
    prat <- Pearson.chisq/rdf
    pval <- pchisq(Pearson.chisq, df = rdf, lower.tail = FALSE)
    c(chisq = Pearson.chisq, ratio = prat, rdf = rdf, p = pval)
}
overdisp_fun(modb)
```

```
##       chisq       ratio         rdf           p
## 115.1593812   0.7382012 156.0000000   0.9940163
```

```
# Hosmer-Lemeshow goodness of fit test with ResourceSelection
# package
hoslem.test(df1$resp, y = fitted(modb))
```

```
##
##  Hosmer and Lemeshow goodness of fit (GOF) test
##
## data:  df1$resp, fitted(modb)
## X-squared = 1.7248, df = 8, p-value = 0.9883
```





Although this linear model fits well and shows there is an increase in avoidance behaviour through time, from Fig. 1 in the main text it is clear there is an apparent threshold in the behavioural response. Guppies exposed to conspecifics that had been infected for fewer than 15 days showed no significant avoidance, whereas those exposed to conspecifics infected for over 15 days showed significance avoidance of both visual and chemical cues. This apparent threshold is investigated further in the 'Post-hoc tests' section.

The change observed in avoidance behaviour corresponds to the predicted change in transmission risk

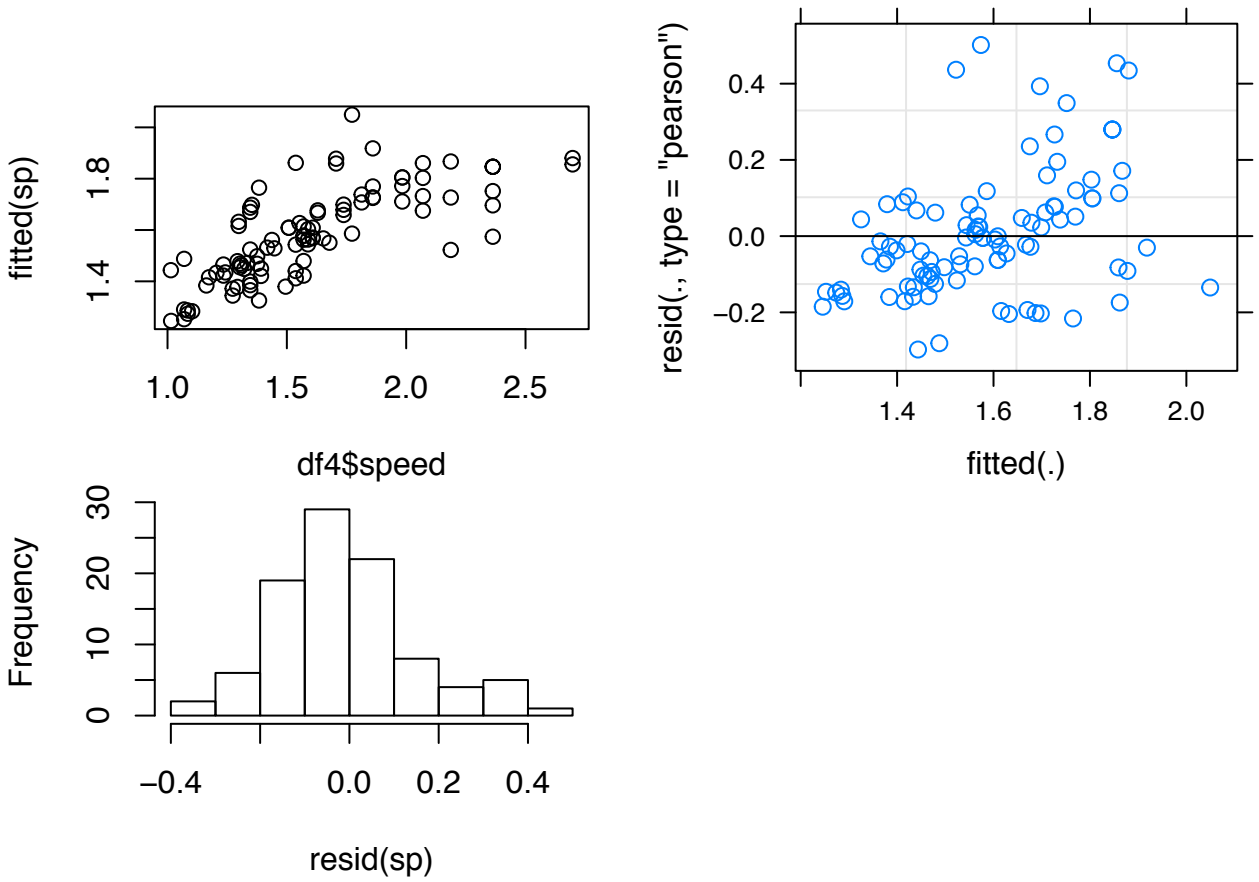
For this analysis we used the data in the archived file 'DatasetS3.csv'. This data sheet includes the following variables:

- **day:** The day of infection on which cues from the stimulus pair were created (chemical) or used (visual).
- **AUC:** The area under the curve of the stimulus fish's infection load over the course of its infection up to day 18 - a measure of its resistance, or ability to limit parasite growth. We have previously shown that transmission is affected by the resistance of the donor (details in the main text), and therefore tested if resistance of an infected conspecific affected how uninfected conspecifics responded to it.
- **intensity:** The number of parasites on the infected stimulus fish on the day on which the stimulus was created (chemical) or used (visual). For trials in which a batch-produced chemical cue was used, we took the maximum individual intensity within that batch.
- **pair:** The identity of the pair of stimulus fish used in a trial - a random effect controlling for repeated measures. Those labelled with a letter were batch-produced cues.
- **speed and transload:** The predicted values of how quickly (in days), and how many parasites would transmit from the infected fish used as stimuli in the behavioural experiment. We used the models constructed using data from a transmission experiment using this system (published as Stephenson et al 2017, Phil. Trans. Roy. Soc. B.) to predict the transmission speed and load from the infection intensity and AUC values, and the day of infection of the stimulus fish. These three variables (intensity, AUC, and day of infection) were the only ones found to explain significant portions of the variation in transmission speed and load.
- **se.speed and se.load:** The standard error associated with the model predictions.
- **resp:** The proportion of time test fish spent associated with the cue of infected conspecific - our response variable.

```
df3 <- subset(df2, speed != Inf)
# removes the fish that were uninfected during the
# behavioural trials and therefore transmission was predicted
# to take an infinite amount of time.
```

```
df4 <- subset(df3, transload < 80)
# removes one outlier prediction of a transmission load of
# ~90 (all others were below 60).

df4$transload.high <- df4$transload + df4$se.load
df4$transload.low <- df4$transload - df4$se.load

df4$speed.high <- df4$speed + df4$se.speed
df4$speed.low <- df4$speed - df4$se.speed

# Testing transmission speed

sp <- glmer(speed ~ day + (1 | pair), data = df4, family = Gamma(link = "log"))
summary(sp)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Gamma  ( log )
## Formula: speed ~ day + (1 | pair)
##    Data: df4
##
##      AIC      BIC   logLik deviance df.resid
##     51.4     61.6    -21.7     43.4       92
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.6159 -0.6158 -0.1344  0.4198  2.7232
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  pair     (Intercept) 0.02149  0.1466
##  Residual             0.03391  0.1841
## Number of obs: 96, groups:  pair, 54
##
## Fixed effects:
##             Estimate Std. Error t value Pr(>|z|)
## (Intercept)  0.575128   0.063099   9.115   <2e-16 ***
## day         -0.009933   0.004630  -2.145   0.0319 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##     (Intr)
## day -0.836
## convergence code: 0
## Model failed to converge with max|grad| = 0.00253098 (tol = 0.001, component 1)
```




```
overdisp_fun(sp)
```

```
##       chisq       ratio         rdf           p
##  2.54760102  0.02739356 93.00000000  1.00000000
```

```
hoslem.test(df4$speed, y = fitted(sp))
```

```
##
##  Hosmer and Lemeshow goodness of fit (GOF) test
##
## data:  df4$speed, fitted(sp)
## X-squared = -1.6443, df = 8, p-value = 1
```

```
# Testing with low and high predicted values
spl <- glmer(speed.low ~ day + (1 | pair), data = df4, family = Gamma(link = "log"))
summary(spl)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Gamma  ( log )
## Formula: speed.low ~ day + (1 | pair)
##    Data: df4
##
##      AIC      BIC   logLik deviance df.resid
##     50.9     61.2    -21.5     42.9       92
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
```

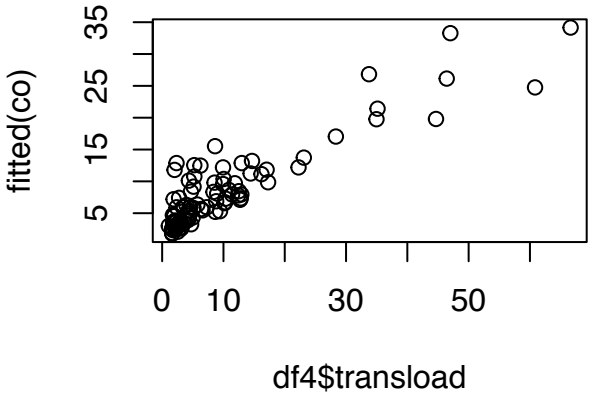
```
## -1.76764 -0.64475 -0.08685  0.47447  2.68093
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  pair     (Intercept) 0.02986  0.1728
##  Residual             0.04210  0.2052
## Number of obs: 96, groups:  pair, 54
##
## Fixed effects:
##              Estimate Std. Error t value Pr(>|z|)
## (Intercept)  0.493194   0.072069   6.843 7.74e-12 ***
## day         -0.013641   0.005231  -2.608  0.00911 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##     (Intr)
## day -0.826
```

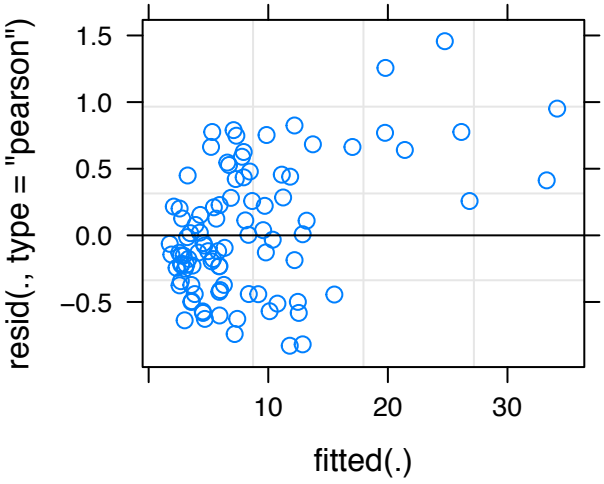
```
sph <- glmer(speed.high ~ day + (1 | pair), data = df4, family = Gamma(link = "log"))
summary(sph)
```

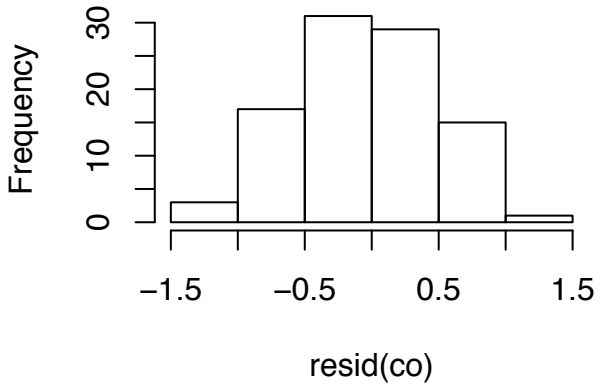
```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Gamma  ( log )
## Formula: speed.high ~ day + (1 | pair)
##    Data: df4
##
##      AIC      BIC   logLik deviance df.resid
##     55.1     65.4    -23.5     47.1       92
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.4521 -0.5482 -0.1707  0.3088  2.8499
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  pair     (Intercept) 0.01647  0.1283
##  Residual             0.02912  0.1706
## Number of obs: 96, groups:  pair, 54
##
## Fixed effects:
##              Estimate Std. Error t value Pr(>|z|)
## (Intercept)  0.651995   0.057126  11.413   <2e-16 ***
## day         -0.007107   0.004235  -1.678   0.0933 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##     (Intr)
## day -0.846
```

```
# Testing transmission load
co <- glmer(transload ~ day + (1 | pair), data = df4, family = Gamma(link = "log"))
summary(co)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Gamma  ( log )
## Formula: transload ~ day + (1 | pair)
##    Data: df4
##
##      AIC      BIC   logLik deviance df.resid
##    566.6    576.9   -279.3    558.6       92
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.3830 -0.5860 -0.1018  0.6931  2.4322
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  pair     (Intercept) 0.4569   0.676
##  Residual             0.3588   0.599
## Number of obs: 96, groups:  pair, 54
##
## Fixed effects:
##             Estimate Std. Error t value Pr(>|z|)
## (Intercept)  0.78410    0.23990   3.268  0.00108 **
## day          0.08044    0.01760   4.570 4.88e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##     (Intr)
## day -0.829
```








```
overdisp_fun(co)
```

```
##      chisq      ratio        rdf          p
## 22.0722851  0.2373364 93.0000000  1.0000000
```

```
hoslem.test(df4$transload, y = fitted(co))
```

```
##
##  Hosmer and Lemeshow goodness of fit (GOF) test
##
## data:  df4$transload, fitted(co)
## X-squared = -7.0337, df = 8, p-value = 1
```

```
# Testing with low and high predicted values
col <- glmer(transload.low ~ day + (1 | pair), data = df4, family = Gamma(link = "log"))
summary(col)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Gamma  ( log )
## Formula: transload.low ~ day + (1 | pair)
##    Data: df4
##
##      AIC      BIC   logLik deviance df.resid
##    537.1    547.4   -264.6    529.1       92
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.4072 -0.6014 -0.0878  0.7212  2.4867
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  pair     (Intercept) 0.4249   0.6519
##  Residual             0.3720   0.6099
## Number of obs: 96, groups:  pair, 54
##
## Fixed effects:
##             Estimate Std. Error t value Pr(>|z|)
## (Intercept)  0.64579    0.24710   2.613  0.00896 **
## day          0.07836    0.01851   4.234 2.29e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

```
## Correlation of Fixed Effects:
##     (Intr)
## day -0.844
```

```
coh <- glmer(transload.high ~ day + (1 | pair), data = df4, family = Gamma(link = "log"))
summary(coh)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Gamma  ( log )
## Formula: transload.high ~ day + (1 | pair)
##    Data: df4
##
##      AIC      BIC   logLik deviance df.resid
##    592.7    602.9   -292.3    584.7       92
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.3630 -0.5637 -0.0912  0.6697  2.3914
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  pair     (Intercept) 0.4781   0.6914
##  Residual             0.3512   0.5927
## Number of obs: 96, groups:  pair, 54
##
## Fixed effects:
##             Estimate Std. Error t value Pr(>|z|)
## (Intercept)  0.90491    0.23589   3.836 0.000125 ***
## day          0.08213    0.01708   4.810 1.51e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##     (Intr)
## day -0.819
```

Post-hoc tests investigating the day 15 threshold

```
# Post-hoc test to see if test fish respond differently to
# pre- and post-day 15 of infection stimulus fish

# First: do their association preferences differ from 50% of
# the time?

df1$cat[df1$dayinf < 15] <- "early"
df1$cat[df1$dayinf >= 15] <- "late"
df1$cat <- as.factor(df1$cat)
summary(df1$cat)
```

```
## early  late
##   123    47
```

```
dfearly <- subset(df1, cat == "early")
dflate <- subset(df1, cat == "late")

t.test(dfearly$resp, mu = 0.5)
```

```
##
##  One Sample t-test
##
## data:  dfearly$resp
## t = -2.5637, df = 122, p-value = 0.01157
## alternative hypothesis: true mean is not equal to 0.5
## 95 percent confidence interval:
##  0.4062923 0.4879532
## sample estimates:
## mean of x
## 0.4471228
```

```
mean(dfearly$resp)
```

```
## [1] 0.4471228
```

```
sd(dfearly$resp)/sqrt(length(dfearly$resp))
```

```
## [1] 0.02062561
```

```
t.test(dflate$resp, mu = 0.5)
```

```
##
##  One Sample t-test
##
## data:  dflate$resp
## t = 3.1646, df = 46, p-value = 0.002753
## alternative hypothesis: true mean is not equal to 0.5
## 95 percent confidence interval:
##  0.5360775 0.6621820
## sample estimates:
## mean of x
## 0.5991298
```

```
mean(dflate$resp)
```

```
## [1] 0.5991298
```

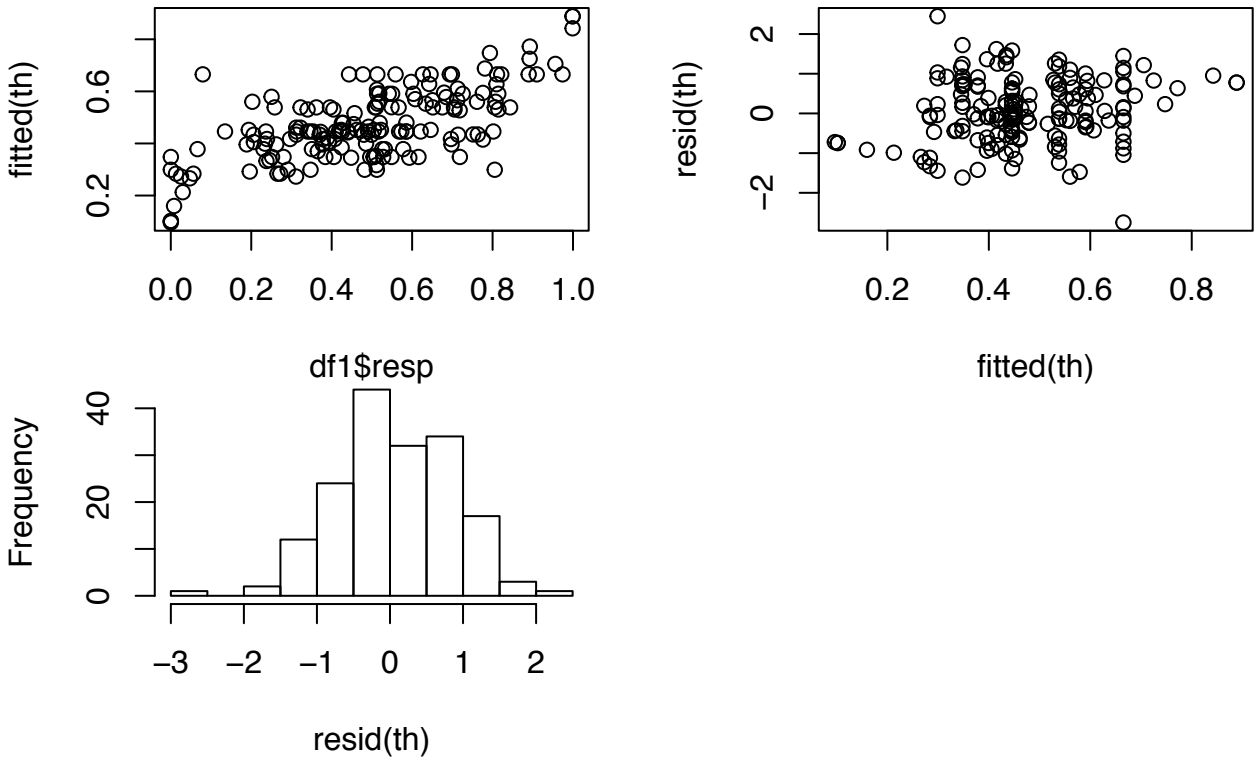
```
sd(dflate$resp)/sqrt(length(dflate$resp))
```

```
## [1] 0.03132415
```

```
# Second: do their association preferences differ when
# exposed to pre- vs post-day 15 of infection stimulus fish,
# or on the cue type available?

th <- glmmadmb(resp ~ cat * sense + (1 | pair), data = df1, family = "beta")
Anova(th)
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: resp
##           Df   Chisq Pr(>Chisq)
## cat        1 15.1504  9.928e-05 ***
```

```
## sense      1  1.0385     0.3082
## cat:sense  1  0.3395     0.5601
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```




```
overdisp_fun(th)
```

```
##       chisq       ratio         rdf           p
## 111.1864566   0.6657872 167.0000000   0.9997131
```

```
hoslem.test(df1$resp, y = fitted(th))
```

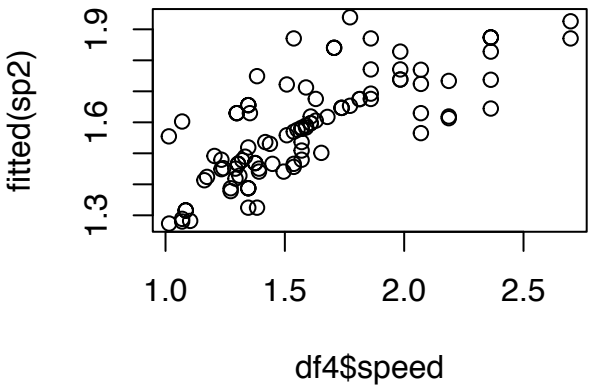
```
##
##  Hosmer and Lemeshow goodness of fit (GOF) test
##
## data:  df1$resp, fitted(th)
## X-squared = 1.495, df = 8, p-value = 0.9928
```

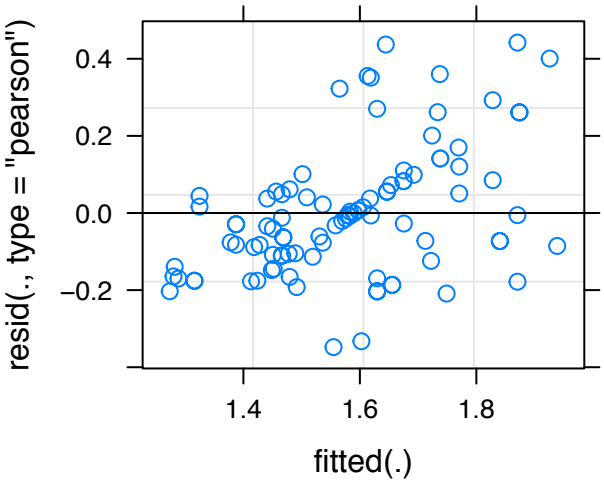
```
# Post-hoc test to see if pre- and post-day 15 stimulus fish
# differ in their predicted transmission speed or load.

df4$cat[df4$day < 15] <- "early"
df4$cat[df4$day >= 15] <- "late"
df4$cat <- as.factor(df4$cat)
summary(df4$cat)
```

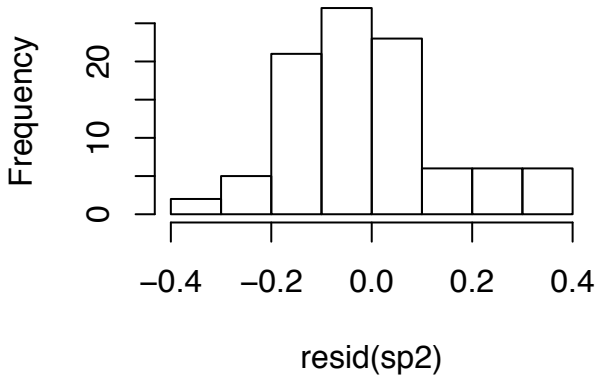
```
## early  late
##    67    29
```

```
# Testing transmission speed

sp2 <- glmer(speed ~ cat + (1 | pair), data = df4, family = Gamma(link = "log"))
summary(sp2)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Gamma  ( log )
## Formula: speed ~ cat + (1 | pair)
##    Data: df4
##
##      AIC      BIC   logLik deviance df.resid
##     55.8     66.1    -23.9     47.8       92
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.86576 -0.59312 -0.07174  0.34426  2.37050
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  pair     (Intercept) 0.02186  0.1479
##  Residual             0.03476  0.1864
## Number of obs: 96, groups:  pair, 54
##
## Fixed effects:
##              Estimate Std. Error t value Pr(>|z|)
## (Intercept)  0.463894   0.037018  12.532   <2e-16 ***
## catlate     -0.006662   0.042829  -0.156    0.876
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##         (Intr)
## catlate -0.302
```








```
overdisp_fun(sp2)
```

```
##       chisq       ratio         rdf           p
##  2.60137960  0.02797182 93.00000000  1.00000000
```

```
hoslem.test(df4$speed, y = fitted(sp2))
```

```
##
##  Hosmer and Lemeshow goodness of fit (GOF) test
##
## data:  df4$speed, fitted(sp2)
## X-squared = -1.6349, df = 8, p-value = 1
```

```
# Testing with low and high predicted values
sp2l <- glmer(speed.low ~ cat + (1 | pair), data = df4, family = Gamma(link = "log"))
summary(sp2l)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Gamma  ( log )
## Formula: speed.low ~ cat + (1 | pair)
##    Data: df4
##
##      AIC      BIC   logLik deviance df.resid
##     57.2     67.5    -24.6     49.2       92
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.00979 -0.67477 -0.04411  0.49024  2.27545
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  pair     (Intercept) 0.03008  0.1734
##  Residual             0.04389  0.2095
## Number of obs: 96, groups:  pair, 54
##
## Fixed effects:
##             Estimate Std. Error t value Pr(>|z|)
## (Intercept)  0.34493    0.04333   7.961 1.71e-15 ***
## catlate     -0.02543    0.04878  -0.521    0.602
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
```

```
##         (Intr)
## catlate -0.292
```

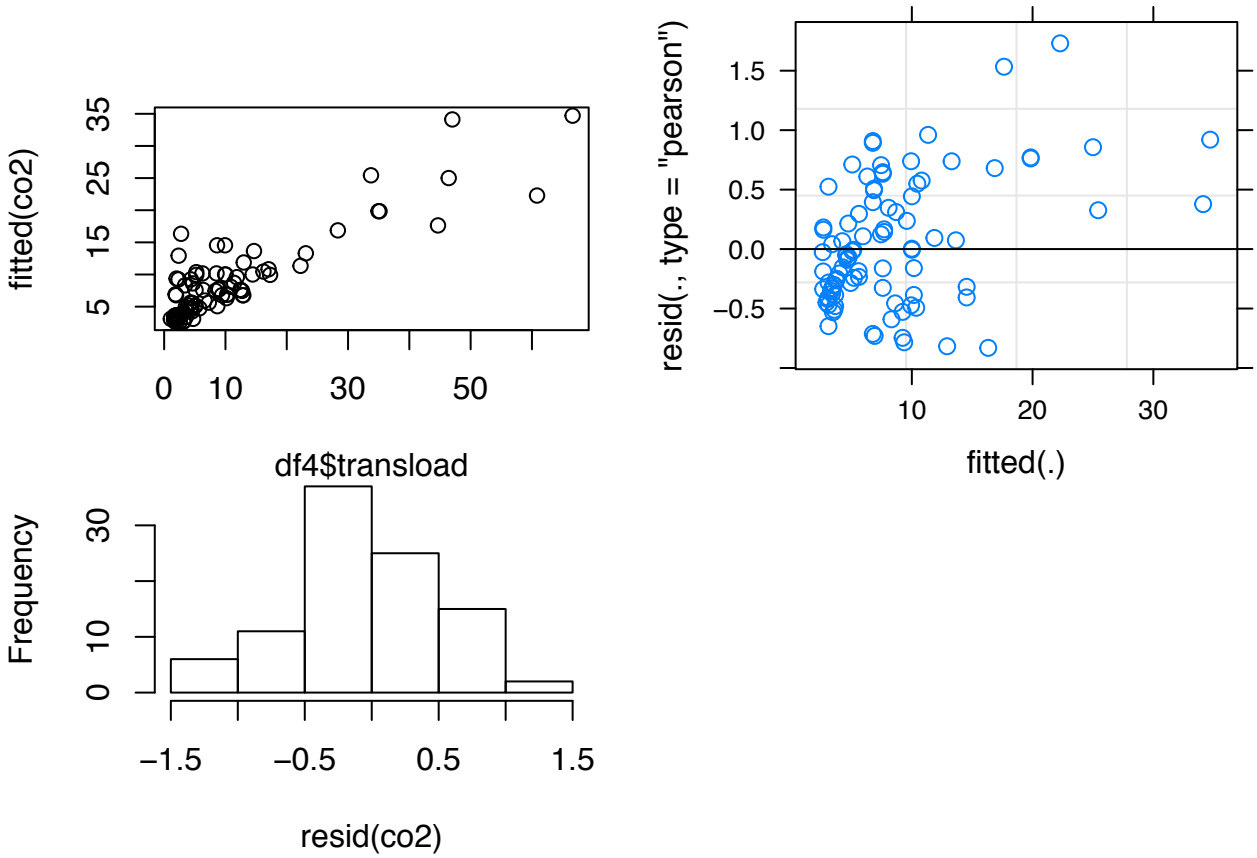
```
sp2h <- glmer(speed.high ~ cat + (1 | pair), data = df4, family = Gamma(link = "log"))
summary(sp2h)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Gamma  ( log )
## Formula: speed.high ~ cat + (1 | pair)
##    Data: df4
##
##      AIC      BIC   logLik deviance df.resid
##     57.8     68.1    -24.9     49.8       92
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.6979 -0.5410 -0.1547  0.2325  2.8729
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  pair     (Intercept) 0.01685  0.1298
##  Residual             0.02935  0.1713
## Number of obs: 96, groups:  pair, 54
##
## Fixed effects:
##             Estimate Std. Error t value Pr(>|z|)
## (Intercept) 0.569131   0.032643  17.435   <2e-16 ***
## catlate     0.007112   0.038877   0.183    0.855
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##         (Intr)
## catlate -0.313
```

```
# Testing transmission load
co2 <- glmer(transload ~ cat + (1 | pair), data = df4, family = Gamma(link = "log"))
summary(co2)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Gamma  ( log )
## Formula: transload ~ cat + (1 | pair)
##    Data: df4
##
##      AIC      BIC   logLik deviance df.resid
##    582.0    592.2   -287.0    574.0       92
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.28737 -0.55314 -0.09676  0.59190  2.68102
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  pair     (Intercept) 0.5300   0.7280
```

```
##  Residual             0.4164   0.6453
## Number of obs: 96, groups:  pair, 54
##
## Fixed effects:
##             Estimate Std. Error t value Pr(>|z|)
## (Intercept) 1.647326   0.001651   997.8   <2e-16 ***
## catlate     0.310917   0.001651   188.3   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##         (Intr)
## catlate -0.001
```




```
overdisp_fun(co2)
```

```
##      chisq      ratio        rdf          p
## 26.0506782  0.2801148 93.0000000  1.0000000
```

```
hoslem.test(df4$transload, y = fitted(co2))
```

```
##
##  Hosmer and Lemeshow goodness of fit (GOF) test
##
## data:  df4$transload, fitted(co2)
## X-squared = -9.84, df = 8, p-value = 1
```

```
# Testing with low and high predicted values
co2l <- glmer(transload.low ~ cat + (1 | pair), data = df4, family = Gamma(link = "log"))
```

```
summary(co2l)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Gamma  ( log )
## Formula: transload.low ~ cat + (1 | pair)
##    Data: df4
##
##      AIC      BIC   logLik deviance df.resid
##    550.9    561.2   -271.5    542.9       92
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.30268 -0.58372 -0.08158  0.62869  2.71409
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  pair     (Intercept) 0.4983   0.7059
##  Residual             0.4275   0.6539
## Number of obs: 96, groups:  pair, 54
##
## Fixed effects:
##             Estimate Std. Error t value Pr(>|z|)
## (Intercept) 1.492092   0.001684   885.8   <2e-16 ***
## catlate     0.278914   0.001685   165.6   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##         (Intr)
## catlate -0.001
```

```
co2h <- glmer(transload.high ~ cat + (1 | pair), data = df4,
    family = Gamma(link = "log"))
summary(co2h)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Gamma  ( log )
## Formula: transload.high ~ cat + (1 | pair)
##    Data: df4
##
##      AIC      BIC   logLik deviance df.resid
##    609.1    619.4   -300.6    601.1       92
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.2736 -0.5406 -0.1053  0.5563  2.6563
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  pair     (Intercept) 0.5508   0.7422
##  Residual             0.4105   0.6407
## Number of obs: 96, groups:  pair, 54
##
```

```
## Fixed effects:
##             Estimate Std. Error t value Pr(>|z|)
## (Intercept) 1.782642   0.001631  1093.2   <2e-16 ***
## catlate     0.335376   0.001631   205.6   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##         (Intr)
## catlate -0.001
```